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Morphology and fine organization of the spermatheca of *Haplotropis brunneriana* (Orthoptera: Pamphagidae)

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ABSTRACT

We investigated the morphology and structure of spermatheca in *Haplotropis brunneriana* (Orthoptera: Pamphagidae) by light and electronic microscopy. The spermatheca can be subdivided into a tubular seminal receptacle and a multiple-coiled spermathecal tube, both of which are composed of cuticular intima, epithelium layer, basal lamina and muscle layer, from inside to outside. The cuticular intima is made up of a heterogeneous endocuticle, a homogeneous exocuticle and an epicuticle, but the proportion of exocuticle in intima of the seminal receptacle is larger than that of the spermathecal tube. The epithelium layer comprises epithelial cells, gland cells and duct cells. The ultrastructural features of the epithelial cells indicated that its function potentially includes support, secretion and absorption. The gland cells potentially fulfil a secretory role indicative of the abundance of mitochondria and microvilli. In gland cells, an extracellular cavity, showing region differences in the seminal receptacle and the spermathecal tube, was lined with microvillus border. The role of duct cell is responsible for forming the secretory ductules, which connect the extracellular cavity with the lumen of the spermatheca through the cuticular intima. These new data contribute to our understanding of the function of the spermatheca of *H. brunneriana*.

Introduction

The spermatheca arised from ectoderm is a secondary organ in reproductive system of female insects. The key roles of the spermatheca are sperm collection and storage during mating, and sperm release for fertilization. Moreover, the spermatheca is a place where morphological and biochemical changes of the male gamete occur, preparing it for successful fertilization (Longo et al., 1993).

There are distinct differences in number, external shape and structure of spermatheca among various insect species. Orthoptera insects have just one spermatheca which is composed of a seminal receptacle and a spermathecal tube with different length. In contrast, the insects in the other orders usually have more than one spermatheca, for example, *Blaps* (Coleoptera) and *Phlebotomus* (Diptera) have two spermathecae (Chapman, 1998); *Aedes aegypti* (Diptera) have three spermathecae (Pascini et al., 2012). Among insects, the external shape of the seminal receptacle exhibits flattened tubular in *Gryon pennsylvanicum* (Hymenoptera) (Paoli et al., 2014), sac-shape in *Platycleis intermedia* (Orthoptera) (Brundo et al., 2011), spherical shape in *Teleogryllus commodus* (Orthoptera) (Sturm, 2008), golf club-like in *Phlebotomus papatasi* Scopoli (Diptera) (Ilango, 2005) and so on. For structure, depending on the insect species, the cell types of the spermathecal epithelium are different.

Numerous studies on the structure of spermatheca in Orthoptera have been reported, such as *Melanoplus sanguinipes* (Acrididae) (Ahmed and Gillott, 1982), *Rhacocleis annulata* (Tettigoniidae) (Viscuso et al., 1996), *Locusta migratoria* (Acrididae) (Lay et al., 1999), *T. commodus* (Grylloidea) (Sturm, 2008) and *Platycleis intermedia* (Tettigoniidae) (Brundo et al., 2011). However, relatively few studies exist on the ultrastructural organization of the *H. brunneriana* spermatheca. Thus, research on the morphology and structure of spermathecal of *H. brunneriana* by means of light and electronic microscopy can extend our knowledge for the function of insect spermatheca in the reproductive biology.

Material and methods

Insects

Healthy insects of *H. brunneriana* were collected from Huo mountain in Linfen, Shanxi province, China. They were fed in the laboratory.

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Light microscopy

Fifteen mated female adults were dissected in a saline solution and the whole spermatheca was taken out and photographed under a stereomicroscope (Nikon.SMZ-1500). They were fixed in Bouin's liquid for 4 h at 4 °C. The tissues were dehydrated in graded alcohol series for 10 min in each bath, and embedded in paraffin. The 7 μm sections were stained with haematoxylin–eosin. Observations and photographs were made by light microscope (Olympus FSX-100).

Scanning electron microscopy (SEM)

Six spermathecae were fixed in 2.5% glutaraldehyde overnight at 4 °C and then washed three times with phosphate-buffered saline (PBS, 20 min each wash). The spermathecae were cut open using a very sharp blade under a dissection microscope. The seminal receptacle and the spermathecal tube of spermathecae were respectively cut lengthwise into several parts. After being dehydrated in a graded ethanol series, samples were treated with 100% acetone for 20 min. The samples were then critical point dried and gold coated by a sputtering device (JEC-1600). Finally, samples were analyzed and photographed using a JSM-7500F scanning electron microscope.

Transmission electron microscopy (TEM)

Six spermathecae were immediately fixed in a freshly 2.5% glutaraldehyde at 4 °C after the insect spermatheca were dissected and then cut into 1 mm^3 at once. After 4 h, being washed with PBS (pH 7.2–7.4) three times and 20 min per wash, samples were post-fixed in 1% osmium tetroxide for 2 h and washed in PBS three times. The preparations were subsequently dehydrated in a graded acetone series and embedded in EPON-812. Ultrathin sections (60 nm) were cut with a glass knife, stained with uranyl acetate and lead citrate, and then photographed with an H-7650 transmission electron microscope.

Results

Morphology

The structure of female genitalia of *H. brunneriana* conforms to the general model of the reproductive organ in orthoptera insects. A pair of ovaries is composed of ovarioles, which open into the lateral oviducts. The lateral oviducts merge in a common oviduct, which opens to the genital chamber. The spermatheca lying in the back of the genital chamber consists of a flattened tubular seminal receptacle and a coiled spermathecal tube (Fig. 1). The spermathecal tube from the genital chamber goes by several roundabouts before entering the seminal receptacle, which often irregularly bends into a sphere (Fig. 1). The total length of the spermatheca is about 2 cm. The seminal receptacle is about 4 mm long when extended and about 1 mm in width at its widest point. The spermathecal tube runs straight for about 16 mm in length with an average calibre of about 0.5 mm.

Histology

The general organization of the seminal receptacle is similar to spermathecal tube. From inside to outside, these two parts are composed of cuticular intima, epithelium, basal lamina and muscle layer (Figs. 2–5). The epithelium includes epithelial cells, gland cells and duct cells. Large number of dark-stained sperms and secretions were found in the lumen (Figs. 2 and 3). The secretory ductules formed by the duct cells, passing through thick intima, were identified in continuous cuticular intima (Figs. 4 and 5). Distinct extracellular cavities appear in the epithelium layer (Figs. 4 and 5). However, the thickness of the epithelium layer in the seminal receptacle is larger than that in the tube (Figs. 4 and 5). The muscle layer, located outside the basal lamina, is composed of circular muscle and longitudinal muscle (Figs. 2 and 3), which are arranged non-uniformly. The circular muscle is dominant in the seminal receptacle section, while the longitudinal muscle is dominant in the tube section (Figs. 2 and 3).

Ultrastructure

In SEM, irregularly arranged folds and secretory ductules' openings appear on the cuticular intima inner surface of the seminal receptacle (Figs. 7 and 8) and the spermathecal tube (Figs. 9 and 10). The openings in the seminal receptacle are about 0.9 μm wide (Fig. 8), which are larger than that (ca. 0.4 μm) in the spermathecal tube (Fig.11).

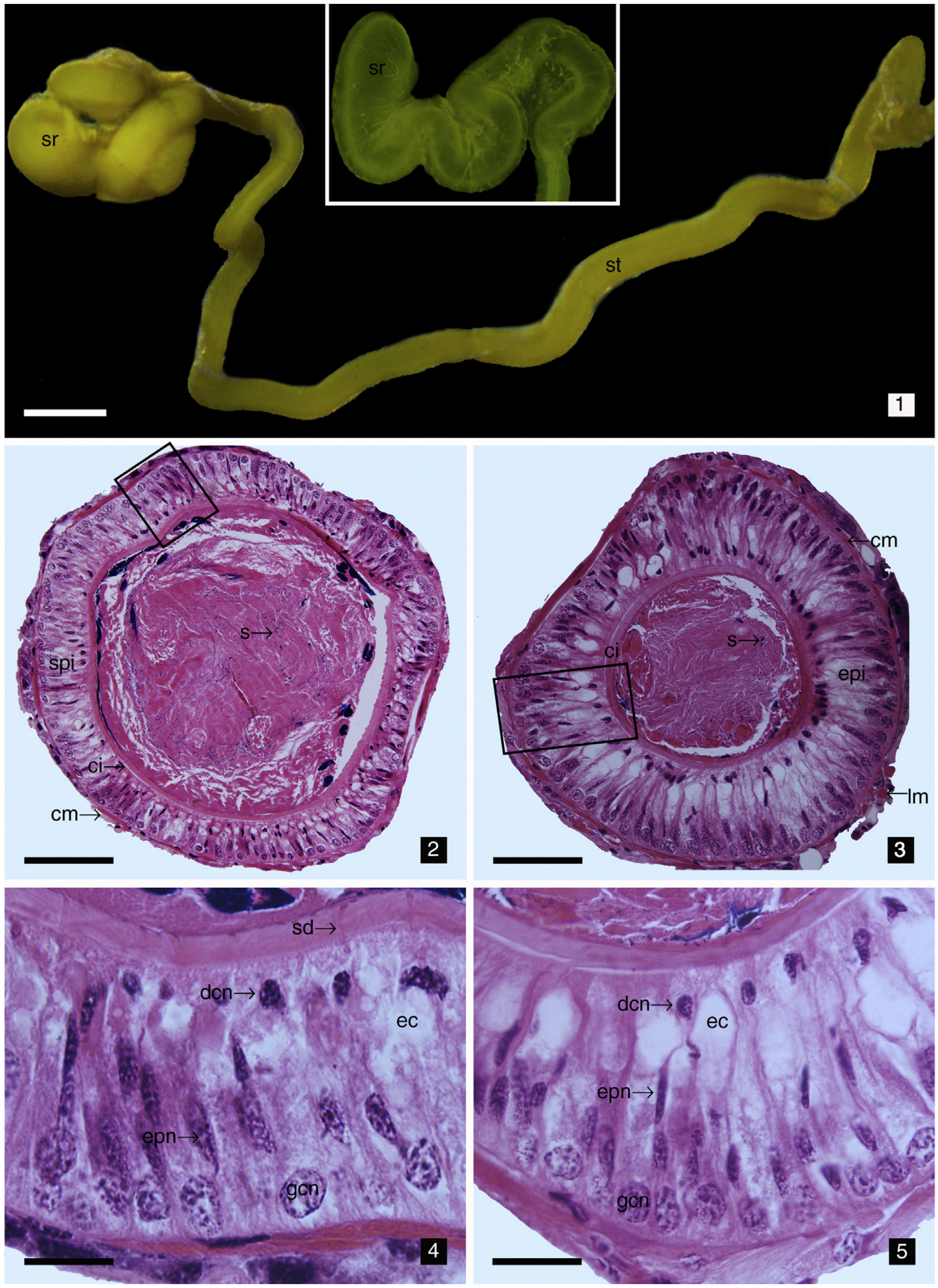
The cuticular intima is made up of a heterogeneous endocuticle, a homogeneous exocuticle and an epicuticle (Figs. 12 and 13). The epicuticle is an osmophilic thin layer, delimiting the spermathecal lumen (Figs. 12 and 13). The proportion of the endocuticle and the exocuticle of intima in the seminal receptacle is imparity to that in the spermathecal tube. The endocuticle and the exocuticle in the intima of the seminal receptacle respectively are about 11 μm and 10 μm thickness (Fig. 12), and the endocuticle has a thickness of about 16 μm and the exocuticle is only 4 μm thickness in the spermathecal tube (Fig. 13).

The epithelial cell with an elongated heterochromatic nucleus is located next to the gland cells (Fig. 14). Mitochondria are rich in the apical cytoplasm of the epithelial cell where they have a microvillus border towards the cuticular intima (Figs. 15 and 16). Some epithelial cells tightly attach to each other and cell junction is also observed (Fig. 16). The individual or grouped microtubules run perpendicular to the cuticular intima interface in the cytoplasm of epithelial cell and extend through the cells from the apical to the basal part (Figs. 16–18).

In contrast to the epithelial cell, the gland cells have oval or spherical nuclei situated in the basal cytoplasm (Fig. 14). The gland cell manifest typical structures for producing proteins: mitochondria, rough endoplasmic reticulum (RER), as well as vesicles of different size and electron-dense granules (Fig. 14). Each gland cell has a large extracellular cavity, about 8 μm × 12 μm in diameter, which is lined with microvilli (Figs. 14, 19 and 20). But there are different characters in alternative regions of the cavities observed under TEM. In the seminal receptacle, this cavity is stained slightly and the vicinity of the cavity is enriched with mitochondria (Fig. 14). In contrast, in the spermathecal tube, the cavity is stained heavily (Fig. 19). There are comparatively fewer mitochondria in the cytoplasm around the cavity, but, vesicles of different electron-density granules are found here (Fig. 19). And most of all, the cavity is filled with homogeneous substance in the spermathecal duct (Fig. 19).

Duct cells show less cytoplasm, possessing heterochromatic nucleus, are frequently arranged adjacent to the extracellular cavity of gland cells (Fig. 21). The duct cells are responsible for forming the secretory ductules, which can be seen in the duct cells and the cuticular intima (Figs. 13, 15 and 21). One end of the secretory ductule is closed off by an end apparatus in the extracellular cavities of the gland cells (Figs. 19 and 22), the other end that goes across the intima (Figs. 13 and 15) opens into the lumen of the spermatheca (Figs. 7 and 8).

These cells lie on a thin, fibrillar basal lamina with wide invaginations. The basal membrane is covered by muscular layer, including circular muscle and longitudinal muscle (Figs. 2 and 3).



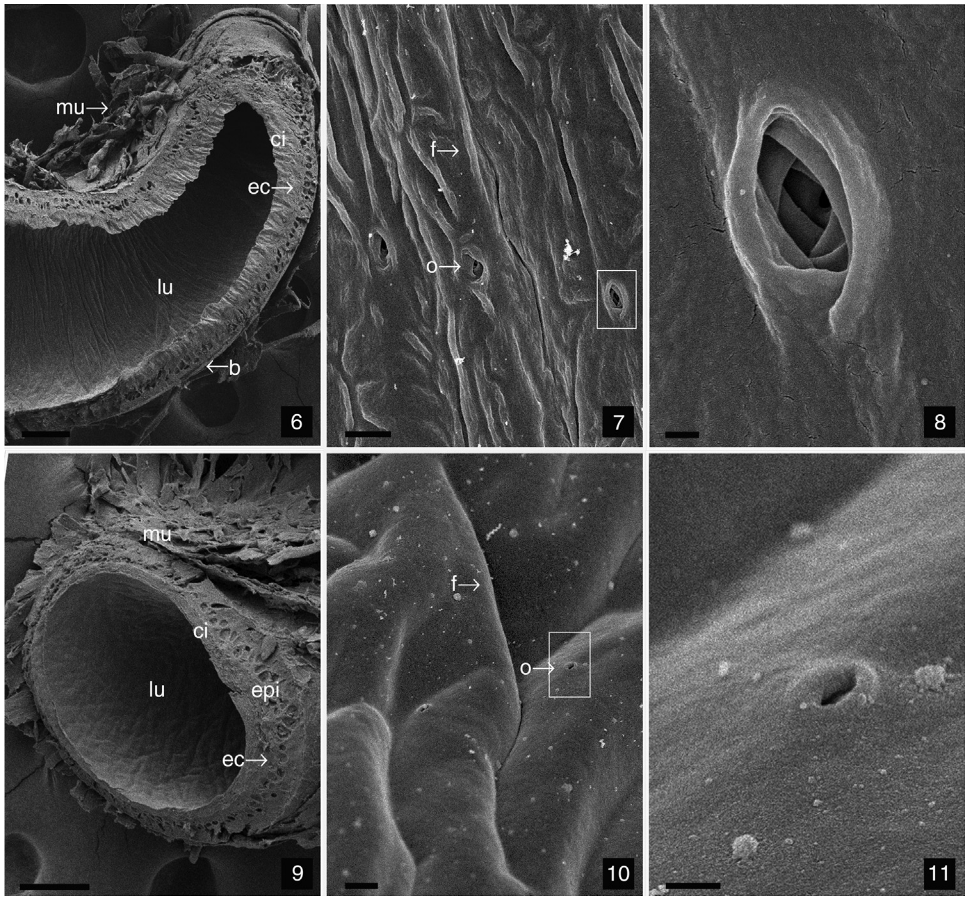
Figs. 1–5. Morphology and microstructure of the spermatheca of *H. brunneriana*. (1) General view of the spermatheca of a mated female composed by seminal receptacle (sr) and the spermathecal tube (st). The inset shows a stretched-out seminal receptacle. Scale bar: 1 mm; (2) the transverse section of seminal receptacle. Scale bar: 260 μm; (3) the transverse section of the spermathecal tube. Scale bar: 130 μm; (4) details of the dilation in little square in 2. Scale bar: 50 μm; (5) details of the dilation in little square in 3. Scale bar: 50 μm. s, sperms; ci, cuticular intima; cmu, circular muscle; lmu, longitudinal muscle; epl, epithelium layer; epcn, epithelial cell nucleus; gcn, gland cell nucleus; dcn, duct cell nucleus; ec, extracellular cavity; sd, secretory ductule.

Discussion

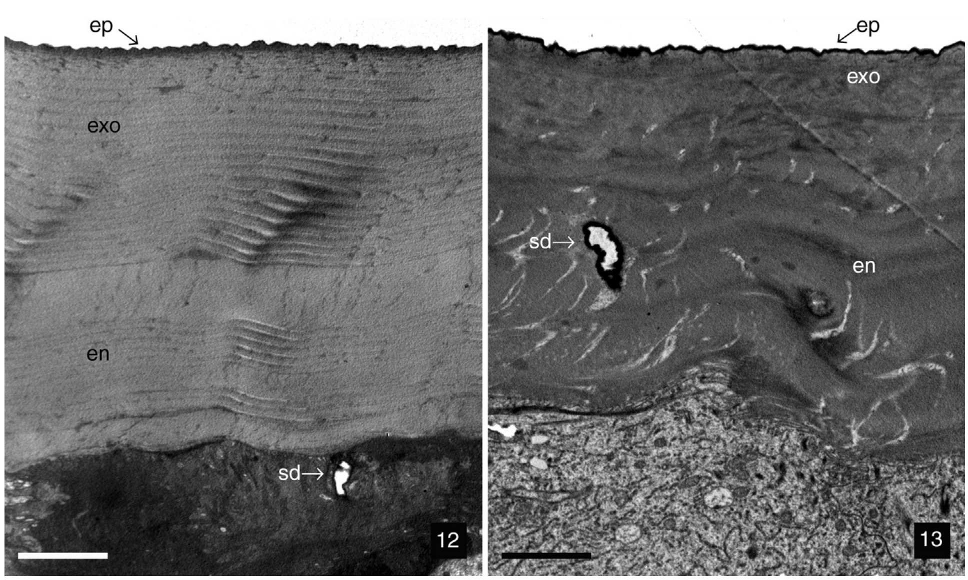
The spermatheca of *H. brunneriana* comprises a seminal receptacle and a spermathecal tube, but the spermathecal gland was not found, as in the vast majority of orthopteran insects (Brundo et al., 2011; Lay et al., 1999; Viscuso et al., 1996).

The position of the spermatheca varies among different insect orders. The spermathecal tube of *H. brunneriana* opens into the genital chamber independently of the oviduct. In contrast, it opens into the dorsal wall of the median oviduct in *Gryon pennsylvanicum* (Hymenoptera, Plastygastridae) (Paoli et al., 2014) or into the vagina in *Melipona bicolor bicolor* (Hymenoptera, Apidae) (Cruz-Landim et al., 2006).

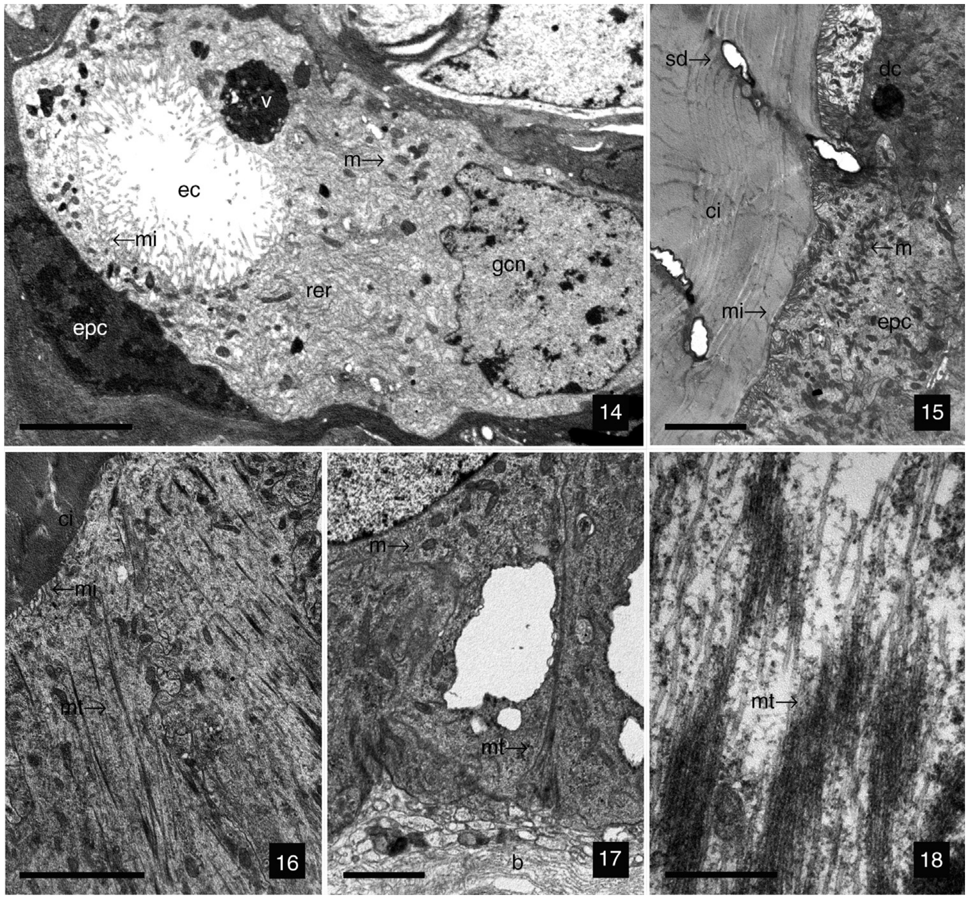
Acanthae were found on the inner surface of the cuticular intima of spermatheca in some insect species. The existing data showed that acanthae are located in three regions: the seminal receptacle, transition region between the seminal receptacle and the spermathecal tube, and the basal spermathecal tube. For instances, in *Oedaleus infernalis* (Acrididae, Oedipodinae) the acanthae were only found in the seminal receptacle (He et al., 2004). In *L. migratoria* (Acrididae, Oedipodinae), they appear in both the transition region and the basal spermathecal tube (Lay et al., 1999). In



Figs. 6–11. Scanning electron microscopy (SEM) showing of cuticular intima of the spermatheca. (6) The longitudinal section of the seminal receptacle. Scale bar: 100 μm; (7) the inner surface of cuticular intima in the seminal receptacle. Scale bar: 2 μm; (8) represents the region delimited by the rectangle in 7. Detail of opening a ductule into the lumen. Scale bar: 200 nm; (9) cross-section through the spermathecal tube. Scale bar: 100 μm; (10) the inner surface of cuticular intima in the spermathecal tube. Scale bar: 2 μm; (11) detail of opening in little square in 10. Scale bar: 500 nm. l, lumen; ci, cuticular intima; epl, epithelium layer; ec, extracellular cavity; mu, muscle; bl, basal lamina; f, folds; o, opening.



Figs. 12 and 13. The ultrastructure of the cuticular intima of the spermatheca in TEM. (12) The cuticular intima of seminal receptacle. Scale bar: 10 μm; (13) the cuticular intima of spermathecal tube. Scale bar: 10 μm. ep, epicuticle; en, endocuticle; ex, exocuticle; sd, secretory ductule.



Figs. 14–18. Epithelial cells and gland cell of the spermatheca in TEM. (14) The epithelial cells, have an elongated heterochromatic nucleus, is located next to the gland cells. Gland cell has a large extracellular cavity. Basal is to the right. Scale bar: 5 μm; (15) apical part of the epithelial cell with microvilli border. Scale bar: 5 μm; (16) the individual or grouped microtubules run perpendicular to the cuticular intima interface. Scale bar: 5 μm; (17) microtubules extend through the cells and end to the basal lamina. Scale bar: 2 μm; (18) detail of the microtubules. Scale bar: 500 nm. ec, extracellular cavity; mi, microvilli; v, vesicles; epcn, epithelial cell nucleus; gcn, gland cell nucleus; mit, mitochondrion; rer, rough endoplasmic reticulum; dc, duct cell; sd, secretory ductule; ci, cuticular intima; epc, epithelial cell; mt, microtubules; cj, cell junction; bl, basal lamina.

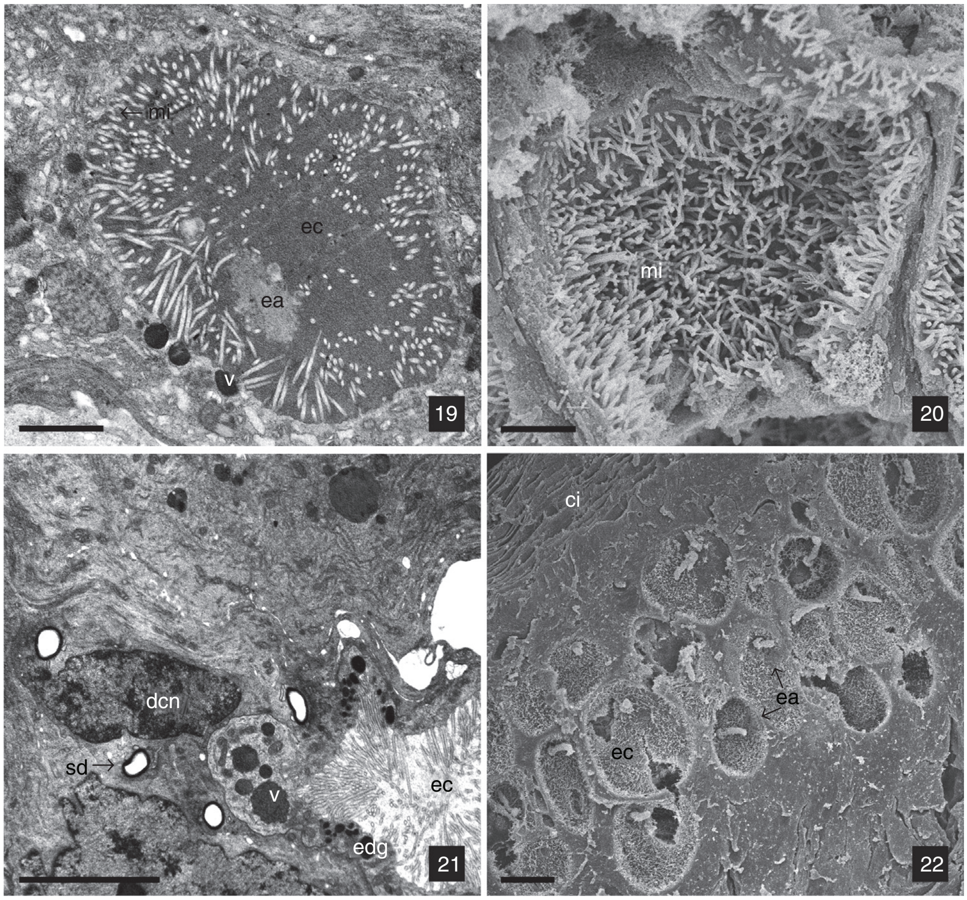
Dichromorpha viridis (Acrididae, Gomphocerinae) they were identified in both the seminal receptacle and the transition region (Johnson and Niedzlek-Feaver, 1998). However, there are not acanthae in the inner surfaces of the spermatheca in *H. brunneriana*, as does that of *Schistocerca americana* (Acrididae, Cyrtacanthacridinae) and *Dissosteira carolina* (Acrididae, Oedipodinae) (Gardner and Niedzlek-Feaver, 2004).

Acanthae distributed on the spermathecal inner surface may perform different functions. Lay et al. (1999) speculated that the function of these acanthae is to keep a distance from the sperm mass to the spermathecal wall, while some authors suggested that they may possibly serve as a mechanical function (Richards and Richards, 1979). There are no acanthae but heavily folds in the inner surface of the seminal receptacle region of *H. brunneriana* (Fig. 7). These folds presumably play a role similar to those acanthae. Further research is necessary for the clarification.

The cuticular intima of insects typically includes 3 layers: endocuticle, exocuticle and epicuticle. Endocuticle with chitin and resilin provide flexibility and elasticity. Large amounts of proteins in exocuticle are tanned, providing hardness for cuticular intima (Wigglesworth, 1948). The proportion of endocuticle and exocuticle in the cuticular intima of *H. brunneriana* showed regional differences (Figs. 12 and 13). Compared to the spermathecal tube, the proportion of endocuticle and exocuticle in the seminal receptacle is smaller. Thus, seminal receptacle with relatively higher hardness is suitable for a stable place to store sperms, and the flexible spermathecal tube is used for mating and releasing sperms.

In general, the cell types of epithelium in insect spermatheca vary among different species. Some only consist of the epithelial cells, as in *Liogryllus bimaculatus* (Gryllidae, Gryllinae) (Sathe and Joshi, 1988); some are made up of the epithelial cells and the gland cells like *L. migratoria* (Lay et al., 1999) and *Oxya chinesis* (Acrididae, Oxiyinae) (Dou and Xi, 2002); and other are composed of the epithelial cells, the gland cells and the duct cells, as reported in *O. infernalis* (Acrididae, Oedipodinae) (He et al., 2004) and *Pararcyptera microptera meridionalis* (Acrididae, Gomphocerinae) (Li et al., 2016). Our study showed that *H. brunneriana* epithelium contains three types of cells: epithelial cells, gland cells and duct cells. The duct cell is a specialized cuticle-forming cell in consideration of its cytological characteristics and functions (Noirot and Quennedey, 1974). Therefore, we suggest that *H. brunneriana* of Pamphagidae have closer relationships with *O. infernalis* or *P. microptera meridionalis* of Acrididae as well as existing three cell types than *L. migratoria* or *Oxya chinesis* of Acrididae with two cell types in phylogenetic relationships, and is far from *Liogryllus bimaculatus* of Gryllidae with only one cell types. Whether we may use this feature as a reference standard for analyzing the systematic relationship among members of the same group, due to the deficiency of data in literature, this topic deserves further studies.

In epithelial cell, the apical cytoplasm contains numerous mitochondria and microvilli (Figs. 15 and 16), it is indicative of cuticular intima formed by active transport. The basal regions possibly involve in the re-absorption or the nutrient transport process from the haemocoel to the epithelium of spermatheca (Fig. 17),



Figs. 19–22. Duct cell and the extracellular cavity of the spermatheca. (19) The extracellular cavity shows homogeneous substances of spermathecal tube in TEM. Scale bar: 10 μm; (20) the large extracellular cavity lined with dense microvilli in SEM. Scale bar: 5 μm; (21) duct cell, less cytoplasm, is arranged adjacent to the extracellular cavity of gland cell. Scale bar: 2 μm; (22) end apparatus can be seen in extracellular cavity of gland cells in SEM. Scale bar: 2 μm. mi, microvilli; ec, extracellular cavity; ea, end apparatus; v, vesicles; sd, secretory ductule; dcn, duct cell nucleus; ci, cuticular intima; e.g., electron-dense granules.

given a large number of mitochondria and invaginations of the basal lamina as referred in other insects (Brundo et al., 2011). In addition, there are many longitudinal microtubules from apical to basal (Figs. 16 and 17) running perpendicular to the microvilli border, which indicated the epithelium was likely involved in providing the mechanical stress during sperm transport.

There are regional differences of extracellular cavity in the spermatheca of *H. brunneriana*, the phenomenon also reported in other species (Lay et al., 1999). In the seminal receptacle, there are mitochondria in the vicinity of the extracellular cavity so we speculate that here may carry out active transport (Fig. 14). In contrast, in the spermathecal tube, it shows few mitochondria, and homogeneous substances were observed in the cavity (Fig. 19), it is also conceivable that substances were secreted from gland cell via the microvilli or merocrine secretion. It is indicated that the gland cells in alternative regions may have the different functions. For *H. brunneriana* the epithelium, the gland cells are connected with the duct cells, so it classified as class 3 according to the Noirot and Quennedey (1974) classification about the epidermal gland cell.

The distribution of the muscles in *H. brunneriana* is irregular. In the seminal receptacle, the circular muscle is dominant, as the longitudinal muscle is in the tube section. This muscle arrangement is conducive to sperm collection and releasing.

In this paper, the morphology and structure of spermatheca of *H. brunneriana* were examined. The results showed that the seminal receptacle and the spermathecal tube have similar organizational structure, but some differences were also identified including the extracellular cavity of gland cell, the proportion of endocuticle and exocuticle in cuticular intima, the distribution of muscle. Thus, it is likely that the seminal receptacle and the spermathecal tube have different biological functions. The seminal receptacle may serve for the collection and storage of the sperm, while the spermathecal tube is potentially responsible for the sperm transport.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

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